Rev 03/10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application	:	Laszlo Takacs et al.
Application No.	:	10/588,392
Filed	:	August 03, 2006
Confirmation No.	:	8285
For	:	MONOCLONAL ANTIBODY BASED BIOMARKER DISCOVERY AND DEVELOPMENT PLATFORM
Examiner	:	Jeffrey S. Lundgren
Attorney's Docket	:	NU-BI-659XX

TC Art Unit: 1639

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DECLARATION OF LASZLO TAKACS, Ph.D. UNDER 37 C.F.R § 1.132

Via Electronic Filing
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Laszlo Takacs hereby declare as follows:

1. At the present time, I hold the positions of Chief Operating Officer and Chief Scientific Officer for Biosystems International SAS, France, which is the Licensee of the above-identified patent application. I am also Professor, Dept. of Human Genetics, University of Debrecen, Hungary, specializing in medical genome biology.

2. My previous responsibilities have included Head of Genome Research, Pfizer, Fresnes Laboratories (France); Research Scientist, Amgen (USA); and Laboratory Head, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health (USA).

3. I am a named author on more that 100 publications in peer reviewed journals, seven of which have been cited by others more than 150 times. I am a named inventor on more than 10 patents.

4. I am a co-inventor of the subject matter described and claimed in the above-identified patent application.

5. I present below the results of a development project carried out by the licensee company, under the direction of my co-inventors and me, since the filing of the instant patent application. This development project goes further than the experiments reported in the above-identified patent application and is an additional example of successful practice of the claimed invention.

A challenge in the treatment of lung cancer has been the lack of tools for ***early, pre-symptomatic detection*** of actual ***clinical symptoms*** of lung cancer ***generally present at advanced stages*** of this disease. I describe here the use of the claimed invention as a widely applicable plasma proteome profiling tool, in this case for the discovery of lung cancer specific biomarkers and early stage lung cancer specific biomarker candidates. This use of the method of the invention is in addition to our previously published use of our invention for the discovery of specific biomarkers in chronic obstructive pulmonary disease. These previously obtained results were presented in the instant patent application as Example I .

5a. **Experiments:** My co-workers and I produced and characterized nascent monoclonal antibody libraries (a total of 3848 hybridomas) according to the invention, which libraries, because of the details of our method, contain monoclonal antibodies directed to the ***natural form*** of protein antigens present in the plasma of early stage lung

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cancer patients. We performed differential plasma profiling of normal and cancer plasma proteomes of four clinical cohorts, totalling 301 patients with early stage lung cancer and 147 healthy controls, via high throughput ELISA screening (see workflow diagram given in Fig. 1 below).

Two different separation strategies were then used to obtain protein fractions of high complexity. We started with multiple-step plasma processing, which was aimed at depleting the plasma of the most abundant proteins and then permitting access to the low-abundant plasma proteins to obtain the complex antigen intended for antibody generation (see Fig.1). First, the most abundant proteins, representing ~90% (w/w) of the pooled plasma from twenty non-small-cell lung cancer patients, were removed by means of a commercially available immunoaffinity depletion column. Next, a portion of the remaining proteins in the abundant protein-depleted sample was enriched in glycosylated proteins, which are known as a source of potential cancer biomarkers (Peracaula et al., 2008), using multi-lectin affinity chromatography (Kullolli et al., 2008).

Both the depleted plasma and the glycosylated protein enriched fractions were then subjected to immuno-affinity chromatography based normalization. (This new technology is the subject matter of a separate patent application, WO 2007/012982 A2.) The normalization step reduces representational differences among the remaining plasma proteins (those originally in low abundance) and maximizes the number of protein species reaching the immunogenic threshold during immunization. Shotgun mass spectrometry coupled to spectral counting analysis (States et al., 2006; Carvalho et al., 2008) indicated that normalization using moderate loading flow on the immunoaffinity column adequately reduced plasma protein representational differences. Consequently, the apparent complexity of the normalized samples, measured as the number of visible bands and/or smears following SDS-PAGE, increased as compared to normal and depleted plasma samples. The final yield after normalization represented 1.2 - 2.5%

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(w/w) of the starting plasma protein input, thus increasing the probability of the less prevalent proteins being capable of stimulating an immune response. Referring again to Fig. 1, the protein fractions obtained were used to immunize two groups of Balb/c mice using standard protocols.

5b. **Results:** We identified thirteen lung cancer specific ($p<0.05$) monoclonal antibodies (mABs) and their cognate protein antigens (candidate biomarkers) that individually were capable of discriminating between early stage lung cancer patients and their controls. A panel of six of the thirteen specifically identified mABs showed an increased diagnostic accuracy in identifying "true positives" over "false positives" (see Fig. 2), having the power of over 82% specificity and 80% sensitivity to identify early stage (Stage I) lung cancer patients in the tested cohorts (see Fig. 2 and the Table). The mABs are specific for lung cancer; they do not detect other cancers or other pulmonary diseases. The majority of the identified mABs detect antigens present in lung cancer cells *in situ also* as well as in plasma. Based on these results, the Licensee company, Biosystems International, has started the development of a new diagnostic tool for the early detection of lung cancer.

6. **Advantages of our claimed method of biomarker development:** By using an aliquot of the abundant protein-depleted complex analyte, e.g., plasma, directly as the immunogen, as described and claimed in the instant application, the method of the invention raises antibodies against the ***natural*** form of the protein antigens present in the complex analyte (i.e., the form of the protein antigens obtained naturally after post-translational processing). ***The method of the invention is extremely efficient at generating high quality biomarker candidates.*** As shown, in this ***one example***, we were able to identify ***thirteen new lung cancer specific monoclonal antibodies and their new***

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cognate protein antigens. Previous to our work, only a few plasma biomarkers for lung cancer at any stage had been identified. These previously identified proteins lack sufficient sensitivity and specificity to be useful for any kind of patient screening (Kulpa et al., 2002), and these proteins have no identified utility for early diagnosis in asymptomatic populations (Molina et al., 2003).

7. I have also read and understood the Office Actions of the Examiner, including the Office Action mailed April 14, 2010.

8. I understand that in the Office Action mailed April 14, 2010, the Examiner has rejected the pending claims as anticipated by Conze et al., *Ann. N.Y. Acad. Sci.*, **996**:222-226 (2003) or as obvious over Conze et al. in combination with certain other prior art references. With respect, the teachings of Conze et al. are completely different from the invention and from the claims of the pending application.

Conze et al. are describing a way to generate a population of monoclonal antibodies against the extracellular domain of a ***known*** protein by overexpressing the ***known*** protein in whole cells (NIH-3T3 cells) and then immunizing mice with the resultant cell line. Thus, the reference does not teach a "complex" analyte mixture as that term is understood by those of skill in the art and as we have described in the patent application. In addition, the "sample" that Conze et al. use as the immunogen is ***NOT*** "***depleted***" of abundant proteins in its own context. In fact, the extracellular region of CDCP1 ***IS*** an "***abundant protein***" in that context. Furthermore, I do not understand the Examiner's comparison of a population of NIH-3T3 cells with plasma. Therefore, I submit that Conze et al. is entirely different from our invention, in purpose, and in detail, and does not teach all of the elements of our invention as it has been claimed. Conze's goal was to produce monoclonal antibodies against a known protein. Our invention aims at discovering multiple markers (which we do

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not know at the time when we start the experiments). This approach is fundamentally different from Conze's at two levels: (i) the invention describes a hypothesis-free method (hence we do not aim at producing monoclonal antibodies against a known protein) and (ii) we compare our candidate monoclonal antibodies by screening plasma samples of disease and control subjects (this element is completely missing from Conze's paper).

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References:

Carvalho et al., 2008, *Genet Mol Res* **7**, 342.

Kullolli et al., 2008, *J Sep Sci* **31**, 2733.

Kulpa et al., 2002, *Clin Chem* **48**, 1931.

Molina et al., 2003, *Tumour Biol* **24**, 209.

Peracaula et al., 2008, *Dis Markers* **25**, 207.

States et al., 2006, *Nat Biotechnol* **24**, 333.

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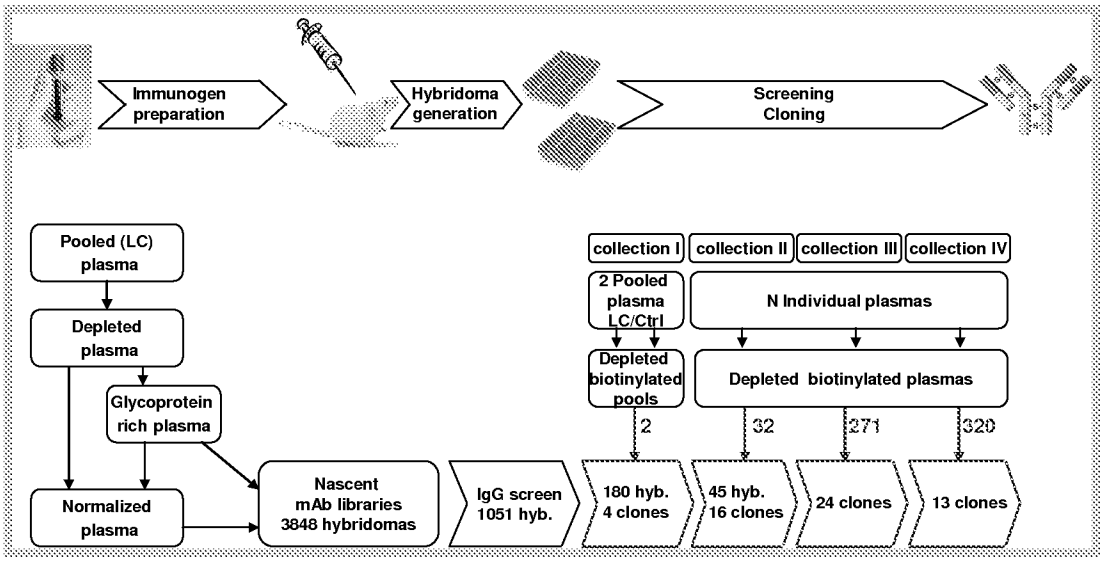

Fig. 1. Lung Cancer biomarker discovery workflow

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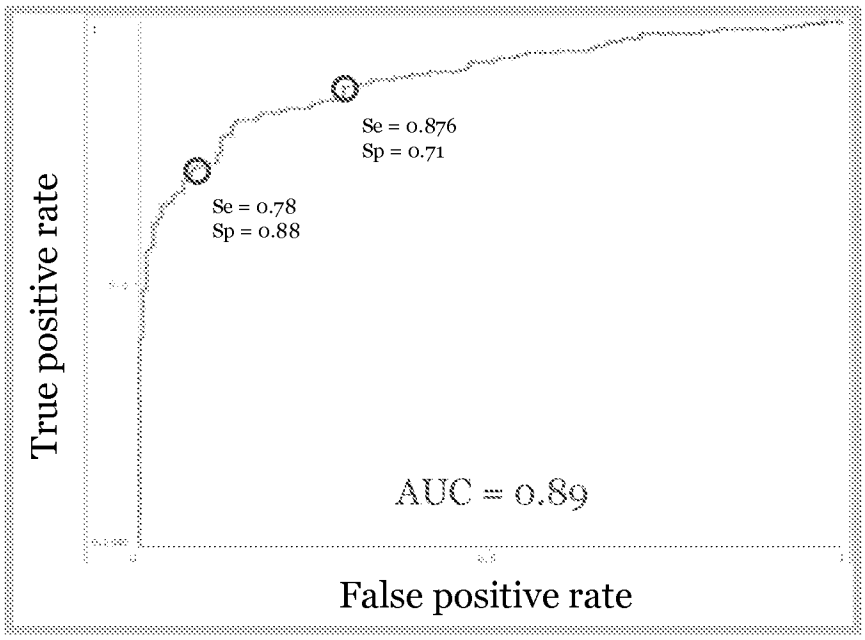

Fig. 2: Clinical performance of a LC specific mAB panel

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Table : Statistical analysis of the screening data obtained from the validated hits with collection III and IV

BSI LC candidates	Collection III			Collection IV	
	LC All stages / Ctrl		LC Stage I / Ctrl	LC All stages* / Ctrl	
Ab name	SN/hybridoma/clone	purified IgG	SN/hybridoma/clone	SN/clone	purified IgG
Bsi0033	$1.15x10^{-04}$	$6.37x10^{-09}$	$1.44x10^{-04}$	$7.94x10^{-05}$	$9x10^{-03}$
Bsi0068	$2.82x10^{-03}$		$1.95x10^{-03}$	$3.67x10^{-02}$	$4.25x10^{-01}$
Bsi0070	$4.14x10^{-12}$	$6.41x10^{-05}$	$4.20x10^{-11}$	$4.54x10^{-02}$	$3x10^{-01}$
Bsi0072	$1.48x10^{-1}$	$6.89x10^{-04}$	$1.95x10^{-01}$	$1.82x10^{-02}$	$1.82x10^{-02}$
Bsi0076	$3.05x10^{-03}$	$3.68x10^{-02}$	$1.82x10^{-03}$	$2.78x10^{-02}$	$3.73x10^{-01}$
Bsi0077	$<10x10^{-19}$	$2.02x10^{-02}$	$2.83x10^{-14}$	$8.49x10^{-04}$	$4.85x10^{-01}$
Bsi0080	$2.02x10^{-03}$		$9.55x10^{-03}$	$2.90x10^{-01}$	$3.66x10^{-04}$
Bsi0270	$7.26x10^{-07}$	$2.50x10^{-05}$	$1.42x10^{-04}$	$2.87x10^{-03}$	$2.34x10^{-03}$
Bsi0272	$5.74x10^{-05}$	$2.75x10^{-07}$	$2.45x10^{-04}$	$3.90x10^{-06}$	$3.70x10^{-06}$
Bsi0349	$3.25x10^{-06}$	$1.12x10^{-05}$	$8.37x10^{-05}$	$1.91x10^{-03}$	$5.54x10^{-01}$
Bsi0351	$1.18x10^{-16}$	$1.01x10^{-09}$	$1.63x10^{-12}$	$6.70x10^{-01}$	$4.48x10^{-02}$
Bsi0352	$3.41x10^{-07}$	$1.31x10^{-07}$	$5.93x10^{-05}$	$4.15x10^{-04}$	$1.16x10^{-02}$
Bsi0358	$2.48x10^{-07}$	$8.90x10^{-13}$	$1.50x10^{-05}$	$1.28x10^{-07}$	$2.83x10^{-02}$
Bsi0359	$1.00x10^{-04}$	$1.28x10^{-05}$	$2.02x10^{-03}$	$6.28x10^{-07}$	$1.93x10^{-01}$
Bsi0392	$1.18x10^{-16}$	$8.50x10^{-13}$	$1.63x10^{-12}$	$1.17x10^{-04}$	$2.04x10^{-02}$

The predictive value of each antibody is represented by the p-value from the statistical test. The biological material used in each experiment is identified as supernatant from nascent hybridomas (SN/hybridoma). supernatant from clonal cell line (SN/clone) and purified IgG.

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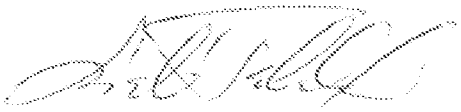
I hereby declare that all statements made herein on personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 28th day of September, 2010.

By 

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